a calmodulin regulation site that with many disease mutations reside proximately. using a variety of spectroscopic methods including CD, fluorescence, NMR, we have determined metal binding affinity, stoichiometry, conformational change and binding modes of Ca^{2+} and Calmodulin.

2089-Pos Board B108
Protein-Protein and Protein-Lipid Interactions Tune Proteorhodopsin Function by Altering Water Dynamics
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Currently, the role of water and the membrane assembly in tuning the function of seven-helical transmembrane (7TM) proteins is not well-understood. Here, we focus on the light activation and functional properties of a prototypical example, the Proteorhodopsin (PR) proton pump from marine bacteria, observing how the protein and surrounding hydration water rearrange upon activation. This is made possible by the application of the powerful residue-specific magnetic resonance methods of electron paramagnetic resonance (EPR), which measures protein segment mobility, and Overhauser dynamic nuclear polarization (ODNP), as recently developed for probing local water diffusivity within 10 Å of a nitroxide spin-label. We investigate further how these dynamics are affected by the surrounding environment, encompassing both protein-protein and protein-lipid interactions.
With these techniques together with optical absorption spectroscopy, we find that water dynamics (both ps scale translational motion and ns scale ''bound'' water) at the membrane protein surface is dramatically affected by the lipid bilayer or surfactant micelle environment. Furthermore, hydration is correlated to functional changes such that water could modulate the timescale of conformational motion. Specifically, the slowdown of translational water motion at the membrane surface, coupled to a lack of bound waters, may facilitate proton uptake by PR.
The association of PR with other PR molecules within the membrane, or oligomerization, has similar functional consequences in addition to effects on the protonation properties of key residues for ion transport (pKa of D97). The implication of our study is that PR-PR association alters the hydrogen bond network within the channel, possibly mediated by an altered interaction with the surfactant and surface hydration water upon oligomerization. This result, combined with the homology of PR with sensory receptors, elicits the intriguing possibility that PR has a functional flexibility mediated by oligomerization.

2090-Pos Board B109
Innovative Tools for the Structural and Functional Investigation of a Multidrug Efflux Pump from Pseudomonas Aeruginosa
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Efflux pumps are macromolecular assemblies that allow for proton-driven transport across both membranes in Gram-negative bacteria. In Pseudomonas aeruginosa, transport is made possible by the reversible assembly of a tripartite protein complex consisting of MexB, a membrane protein responsible for the active transport (energized by the proton motive force), MexA, a periplasmic protein whose putative role is to stabilize the whole complex and OprM an exit channel composed of a ß-barrel inserted in the outer membrane and of a bundle of α-helices running along the periplasmic space.
Such complexes are involved in the resistance against antibiotics, a field where new tools are needed for a better understanding of substrate transport. We have decided to investigate these pumps on two front lines.
First, we have set up methodological developments for the design and production of a new set of synthetic scaffolds (dubbed α-reps for ''artificial alpha repeat protein'') as membrane protein stabilizers and crystallization ortheses. Such interactants are selected through in vitro screening of the membrane proteins targets. We make use of amphipathic polymers (amphipols) to stabilize and immobilize the proteins onto solid support so that the library of possible interactants can be screened.
As a complementary approach, we are working on the functional reconstitution of the pumps into proteoliposomes. Very recently we have designed a functional test for MexB. This original activity assay uses bacteriorhodopsin (BR), a light-activated proton pump, to generate a tunable, robust and reversible proton gradient. In this system, upon illumination with visible light, the photo-induced proton gradient created by the BR is shown to be coupled to the active transport of substrates through the pump. We are now working on the reconstitution of the whole efflux pump.

2091-Pos Board B110
The PLM Homotetramer has a Structural Basis that Parallels that of PLB: The Leucine Zipper
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Phospholemman (PLM or FXYD1) interacts with and inhibits the sodium potassium ATPase (mainly by reducing its Na affinity), an effect that is relieved by PLM phosphorylation. This is analogous to phospholamban (PLB) regulation of SERCA. Like PLB, PLM is thought to also form homo-oligomers, although the structural basis for this oligomerization is still unknown. Here we use both a computational and FRET approach to address this. Alanine substitutions of leucine and isoleucine residues in the PLM transmembrane segment were examined for their effect on PLM-PLM FRET. We found that substitutions at I23, I26, L30 and L33 all significantly reduce FRET, but not so for I29 and L36. In parallel experiments, we used Rosetta to model the PLM oligomer. Mapping of experimental data onto Rosetta models favored tetramer configuration for this oligomer rather than a trimer or pentamer. In the tetramer model, the I23, I26, L30 and L33 residues all face and interact with an adjacent PLM subunit, whereas the I29 and L36 residues face the center of the tetramer and appear unlikely to be involved in the stabilization of the tetramer structure. Additionally, the core of the tetramer is lined with hydrophobic residues and is spatially constricted, suggesting that the PLM tetramer does not function as a channel. We conclude that the PLM homo-oligomer is a tetramer with a structural basis that parallels that of PLB: the leucine zipper.

2092-Pos Board B111
Identifying the Pathways to Permeation through OccD1 in the Outer Membranes of P. Aeruginosa
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Pseudomanas aeruginosa (PA) is a pathogenic Gram-negative bacterium that causes infections, which can often be fatal in hosts with compromised defence mechanisms. PA is difficult to combat due to its resistance to antibiotics. This resistance arises as a consequence of a number of factors including the presence of multidrug efflux pumps and the low permeability of the outer membrane. When designing drugs that target PA, it is imperative to consider how they will gain entry into the cell. Specific beta-barrel proteins control movement of large solutes through the outer membrane; due to the substrate-specific nature of these proteins, drug selection and design is not a straightforward process.
We have performed modelling, docking and simulations to gain some insights into the origins of the substrate-specificity and the pathways of permeation through the OccD1 protein found in the outer membranes of PA. A combination of computational and experimental data allows us to predict the molecular interactions that lead to the preferential recognition of arginine and then to demonstrate how this can be modified by designing a mutant protein that preferentially binds glutamate. Extended simulations enable us to identify additional potential binding sites along the barrel, allowing us to build up a picture of the pathway taken by arginine to permeate through OccD1.

2093-Pos Board B112
Conformational Stability and Substrate Translocation - A Computational Study of the Leucine Transporter
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The human monoamine transporters are involved in a variety of disorders and thus important medicinal targets.[1] There are no available high-resolution structures of the transporters. The leucine transporter (LeuT) is a bacterial homolog of the human monoamine transporters with a sequence similarity of 20-24 %.[2] LeuT has been crystallized with a number of different ligands and in several different conformations. Most recently, an inward facing conformation was solved.[3] The crystallization process required the use of four point mutations as well as the association of an antibody. We have applied computational methods to investigate whether the conformation of this manipulated protein is relevant for the wild-type transporter in a membrane environment. We have performed several molecular dynamics simulations of both the mutated and wild-type transporter, and analyzed the stability through measurements of helix tilt angles. The simulations reveal that the mutant and wild-type systems types behave similarly and both maintain a relatively stable inward facing conformation. The crystal structure of LeuT in an inward facing conformation does not contain any ligands or ions. To investigate the release of substrate and ions from the transporter, we have also performed simulations of wild-type LeuT in the inward facing conformation with sodium and either leucine or